Available online at www.sciencedirect.com

ScienceDirect

Biochimica et Biophysica Acta 1783 (2008) 203–213

Biochimica et Biophysica Acta BBA

www.elsevier.com/locate/bbamcr

# Functional characterisation of the *Schizosaccharomyces pombe* homologue of the leukaemia-associated translocation breakpoint binding protein translin and its binding partner, TRAX

Alessa Jaendling, Soshila Ramayah, David W. Pryce, Ramsay J. McFarlane *

*North West Cancer Research Fund Institute, University of Wales Bangor, Bangor, Gwynedd, LL57 2UW, United Kingdom*

Received 9 May 2007; received in revised form 10 September 2007; accepted 25 October 2007
Available online 7 November 2007

**Abstract**

Translin is a conserved protein which associates with the breakpoint junctions of chromosomal translocations linked with the development of some human cancers. It binds to both DNA and RNA and has been implicated in mRNA metabolism and regulation of genome stability. It has a binding partner, translin-associated protein X (TRAX), levels of which are regulated by the translin protein in higher eukaryotes. In this study we find that this regulatory function is conserved in the lower eukaryotes, suggesting that translin and TRAX have important functions which provide a selective advantage to both unicellular and multi-cellular eukaryotes, indicating that this function may not be tissue-specific in nature. However, to date, the biological importance of translin and TRAX remains unclear. Here we systematically investigate proposals that suggest translin and TRAX play roles in controlling mitotic cell proliferation, DNA damage responses, genome stability, meiotic/mitotic recombination and stability of GT-rich repeat sequences. We find no evidence for translin and/or TRAX primary function in these pathways, indicating that the conserved biochemical function of translin is not implicated in primary pathways for regulating genome stability and/or segregation.


*Keywords:* Translin; Trax; *Schizosaccharomyces pombe*; Genome stability

## 1. Introduction

Chromosomal translocations are associated with carcinogenesis [1,2]. Studies of the breakpoints of lymphoma and leukaemia-associated translocation junctions revealed a DNA consensus sequence which binds a novel, highly conserved protein termed translin, implicating this protein in oncogenic translocation formation [3]. Subsequently, translin binding sequences have been identified in other cancer-associated translocation breakpoints [4–8], human male meiotic recombination hot spots [9] and other human chromosomal rearrangement breakpoints [10–12]. Whilst these findings are largely based on *in vitro* gel mobility shift assays they have resulted in proposals suggesting that translin functions to control genome stability.

Translin was independently identified as the testis brain RNA-binding protein (TB-RBP) [13] and has been implicated in mRNA metabolism, particularly in neurones and in the testis [13–21]. In support of a role for translin in neuronal mRNA processing mice and fruit flies defective in translin exhibit a range of neurological and behavioural problems [22–24]. Collectively these observations indicate that translin may function in more than one important biological pathway.

Translin binds to both RNA and DNA [25–29] and forms an octomeric toroidal structure [25,30], which has similarities to the structures of other protein complexes associated with DNA replication, repair and recombination [31]. It has a high affinity for single-stranded microsatellite GT repeats, d[GT]$_n$, and G-strand telomeric repeats, d[TTAGGG]$_n$, but not corresponding double-stranded DNAs [32,33]. This suggests a role for translin in microsatellite repeat/telomere regulation, although fission yeast recombinant translin protein possesses higher affinities for RNA [GU]$_n$ and [GUU]$_n$ repeats, implying a role in regulation of RNA metabolism, rather than DNA metabolism, in this simpler eukaryote [34]. In addition to this, translin has RNAse, but not

* Corresponding author. North West Cancer Research Fund Institute, College of Natural Sciences, Bangor University, Memorial Building, Deiniol Road, Bangor, Gwynedd, LL57 2UW, United Kingdom. Tel.: +44 1248 382360; fax: +44 1248 370731.
*E-mail address:* ramsay@sbs.bangor.ac.uk (R.J. McFarlane).

0167-4889/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.bbamcr.2007.10.014

DNAse, activity *in vitro* [35]. This is consistent with many lines of evidence suggesting translin regulates mRNA metabolism in higher eukaryotes.

Translin has a binding partner protein termed translin-associated protein X (TRAX) [36]. Loss of translin results in depletion of TRAX from mouse embryonic fibroblasts (MEFs) [22,37] and *Drosophila* cells [38], indicating that translin functions to mediate stable levels of intracellular TRAX in higher eukaryotes. Murine translin and TRAX have functional nuclear export and localisation signals respectively, and nuclear levels of both proteins exhibit interdependence in mouse spermatocytes [39]. TRAX inhibits translin's RNA-binding ability *in vitro*, but enhances its ability to bind specific single-stranded DNAs [40]. Together these findings suggest that TRAX–translin interactions modulate functional specificity.

Both TRAX and translin have been implicated in the regulation of cell proliferation. Translin-deficient MEFs have a reduced cell proliferation rate, although TRAX protein is depleted in these cells, despite TRAX mRNA levels being normal, and so the effect of loss of translin might be indirect [22,37]. Reduction in TRAX levels in HeLa cells resulted in a reduced level of proliferation, adding credence to the possibility that the reduced levels of proliferation in translin-deficient cells is due to reduced levels of TRAX protein [41]. However, over expression of translin in a range of cells resulted in accelerated proliferation indicating that translin may also have a direct influence on cellular proliferation [42].

Other than the association with breakpoint sequences, several lines of evidence also implicate translin and TRAX functions in maintaining genome stability, particularly in response to DNA damage. Firstly, murine translin interacts with GADD34, a protein implicated in the DNA damage response [43]. Secondly, treatment of HeLa cells with the chemotherapeutic DNA damaging agents mitomycin C and cisplatin results in an elevation in the levels of nuclear translin [25]. Thirdly, TRAX has been identified as a DNA damage-dependent interacting partner of the C1D protein [44] which regulates homologous recombination (HR) and non-homologous DNA end joining (NHEJ) [45].

Whilst translin and TRAX have been implicated in DNA damage recovery, recent work by Clauβen et al. [38] found that translin and TRAX defective *Drosophila* embryos had no increased sensitivity to ionizing irradiation and that meiotic crossing over was normal. These findings, and the observation that translin-deficient MEFs show no sensitivity to DNA damaging agents [37], argued against a role for translin and TRAX in DNA damage recovery. Here we employ the facile fission yeast model to extensively analyse the role of translin and TRAX in regulating cell growth, DNA damage response, NHEJ, HR and GT repeat stability. We find no evidence to implicate translin or TRAX as primary regulators of these biological processes; which, to some extent, differs to observations in metazoans. We also find that the regulatory controls on TRAX stability by translin are apparent in this lower eukaryote suggesting that this fundamental process is highly conserved in all eukaryotes and is thus unlikely to be related to tissue-specific functions or cell proliferation functions under normal laboratory conditions.

## 2. Methods

### 2.1. Schizosaccharomyces pombe strains, plasmids and antibodies

A list of strains employed in this study and their genotypes are shown in Table 1. Culture media, strain storage and *S. pombe* transformation was as described by Moreno et al. [46]. Plasmid pade6-469 is described in detail by Szankasi et al. [47]. Plasmid pFY20 is described in detail by Li et al. [48].

Plasmid pAJ1 was made by cloning a PCR fragment containing the $tsn^+$ open reading frame (orf) so it was under the regulation of the *nmt* (*no message in thiamine*) promoter of the pREP3X *S. pombe* vector [49]. The $tsn^+$ gene was amplified using Phusion high fidelity polymerase (Finnzyme). PCR primers contained integral *Bam*HI restriction sites for cloning. The PCR primer sequences were 5′-CGCGGATCCGCGATGAATAAATCAATATTTATTCCG-3′ and 5′-CGCGGATCCGCGTTAAACCAATTTATGTATCCG-3′. *S. pombe* genomic DNA was used as PCR template and the $tsn^+$ orf within pAJ1 was checked by DNA sequencing.

Plasmid pSRSB3 was made by cloning a PCR fragment containing the $trax^+$ orf so it was under the regulation of the *nmt* promoter on the pREP4X vector

Table 1
*S. pombe* strains used in this study

| Strain | Genotype | Source |
|---|---|---|
| BP11 | $h^-$ *ade6-M26* | McFarlane collection |
| BP90 | $h^-$ *ade6-M26 ura4-D18 leu1-32* | McFarlane collection |
| BP420 | $h^-$ *pro1-1* | McFarlane collection |
| BP572 | $h^-$ *ade6-3006 ura4-D18 leu1-32 spc1::ura4*$^+$ | McFarlane collection |
| BP621 | $h^+$ *ura1-61* | McFarlane collection |
| BP685 | $h^-$ *ade6-704 ura4-D18 leu1-32 lig4::kanMX6* | McFarlane collection |
| BP743 | $h^-$ *rad3-136* | McFarlane collection |
| BP1023 | $h^+$ *ade6-52 ura4-D18 leu1-32* | This study |
| BP1079 | $h^-$ *ade6-M26 ura4-D18 leu1-32 tsn::kanMX6* | This study |
| BP1089 | $h^-$ *ade6-M26 ura4D18 leu1-32 trax::kanMX6* | This study |
| BP1162 | $h^+$ *ura4-D18 leu1-32 bub1::kanMX6* | McFarlane collection |
| BP1201 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pSRSB3) | This study |
| BP1205 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pREP4X) | This study |
| BP1220 | $h^-$ *ade6-((GT)*$_8$*-1397) ura4-D18 leu1-32 trax::kanMX6* | This study |
| BP1243 | $h^-$ *ade6-((GT)*$_8$*-1397) ura4-D18 leu1-32 tsn::kanMX6* | This study |
| BP1244 | $h^-$ *ade6-((GT)*$_8$*-1397) ura4-D18 leu1-32* | This study |
| BP1267 | $h^-$ *ade6-M26 tsn::kanMX6* | This study |
| BP1269 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pREP3X) | This study |
| BP1271 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pAJ1) | This study |
| BP1282 | $h^+$ *ade6-52 ura4-D18 leu1-32 tsn::kanMX6* | This study |
| BP1288 | $h^+$ *arg1-14* | McFarlane collection |
| BP1390 | $h^-$ *ade6-M26 ura4-D18 leu1-32 tsn::kanMX6* (pade6-469) | This study |
| BP1391 | $h^-$ *ade6-M26 ura4D18 leu1-32 trax::kanMX6* (pade6-469) | This study |
| BP1345 | $h^-$ *pro1-1 tsn::kanMX6* | This study |
| BP1346 | $h^+$ *ura1-61 tsn::kanMX6* | This study |
| BP1348 | $h^+$ *arg1-14 tsn::kanMX6* | This study |
| BP1455 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pREP3X, pREP4X) | This study |
| BP1456 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pAJ1, pSRSB3) | This study |
| BP1461 | $h^-$ *ade6-M26 ura4-D18 leu1-32* (pade6-469) | This study |
| BP1645 | $h^-$ *mts3-1 ade6-M210 arg3 his3 leu1-32 ura4-D18* | McFarlane collection |
| BP1668 | $h^+$ *tsnΔ mts3-1 ade6 arg3 his3 leu1-32 ura4-D18* | This study |

[49]. The fragment was amplified and cloned as described above for *tsn*$^+$ using primers with an integral *Bam*HI restriction site. The primer sequences were 5′-CGCGGATCCATGTGGACCGTAATCGTTTCTCCTCGGGAG-3′ and 5′-CGCGGATCCTTATGGAAGAGGAATTCCTCTCATTTAAAAATTTTTTA-CAG-3′. The *trax*$^+$ gene within pSRSB3 was checked by DNA sequencing.

Anti-Tsn polyclonal antibodies were raised out of house (Eurogentec, Liege, Belgium) in rabbit via inoculation with synthetic peptides EQSRNENLQEKEHGL and LKNDSLRRHFDGLKY corresponding to residues 45–58 and 204–218 respectively of the translation of *S. pombe* gene SPAC30.03c. Anti-Trax polyclonal antibodies were raised out of house (Eurogentec, Liege, Belgium) in guinea pig via inoculation with synthetic peptides SDGFPLPKDFDRTSI and VDTATPPEEK-RLRST corresponding to residues 46–60 and 217–231 respectively of translation of *S. pombe* gene SPCC736.09c. Both serum sets were affinity purified prior to use.

### 2.2. Gene deletions

Full orf deletions of the *S. pombe tsn*$^+$ and *trax*$^+$ genes were made using the method of Bähler et al. [50]. The primers used for the *tsn*$^+$ orf deletion were 5′-TTATTTGCATACTGAAAACATCATTCGAATATCAACACTACTCAA-CAGCATACATTACAGATTAAGTCGACGGATCCCCGGGTTAATTAA-3′ and 5′-ATATTAAAAAAGCAATTTTATCGGCTCAATTTTAGTCAAGCGTA-CAGCTGGCAAATAAATTGTTAGCAATGAATTCGAGCTCGTTTAAAC-3′. The primers used for the *trx*$^+$ orf deletion were 5′-TATAGACTTATACATT-TATACCTTCCACACGGCTTTGCTGAATTGAGGATATTATAAAACTT-TAACCGAATTTGCCAAATCGGATCCCCGGGTTAATTAA-3′ and 5′-ATTATGATTTTCAAAAGCTGCAAAACAGAAAAACTTTTAA-TAAACTAGTAAGGTGTCTGTCGAGAGCTGTCGATCATATAGAATTC-GAGCTCGTTTAAAC-3′. Correct deletions were confirmed by PCR and Southern blotting.

### 2.3. Fluctuation analyses

Fluctuation tests were carried out as described by Lea and Coulson [51]. Exponentially growing cultures of the strain to be tested were plated onto appropriate solid medium (selective medium was used for strains carrying plasmids) and incubated at 30 °C until micro colonies were visible. For each test seven or nine micro colonies were picked and inoculated into 5 ml of liquid medium (selective medium was used for strains carrying plasmids). These cultures were incubated with rotation at 30 °C until early stationary phase. They were then subjected to serial dilution and higher concentrations were plated onto solid minimal medium (NBA) lacking adenine to measure for adenine prototroph numbers within the culture; higher dilutions were plated onto identical minimal medium plates with supplementary adenine (100 μg/ml) to measure viable cell numbers within the culture. Plates were incubated at 30 °C and colonies counted. A minimum of three duplicates of each set were carried out and the mean values of the median value for each set were used for statistical analysis.

### 2.4. Meiotic crosses

Cultures were grown in yeast extract liquid (YEL), supplemented with 100 μg/ml adenine, to a density of approximately $2.5 \times 10^7$ cells/ml. 600 μl of each strain to be mated was added to a sterile microfuge tube, pulse centrifuged and aspirated. Cell pellets were washed with 1 ml sterile $dH_2O$ and finally resuspended in 20 μl $dH_2O$. Suspensions were spotted onto fully supplemented synthetic sporulation media (SPA) plates and incubated at 30 °C for 3–4 days. After incubation, sporulating cells were scrapped into a microfuge tube containing 1 ml of 0.6% β-glucuronidase® (Sigma)/$dH_2O$ solution and incubated for 16 h at 25 °C. After incubation spores were harvested and resuspended in 30% ethanol and incubated at room temperature for no longer than 5 min. Suspensions were then centrifuged and aspirated dry and cell pellets were resuspended in 1 ml sterile $dH_2O$.

### 2.5. Determination of recombination frequencies

Intragenic recombination frequencies at the *ade6* locus were determined as previously described [52].

To determine intergenic recombination frequencies using prototrophic markers, serial dilutions of spore suspensions were plated onto yeast extract agar (YEA) plates to a colony density of approximately 50–100 colonies/plate. These were then replica-plated onto nitrogen base agar (NBA) plates with and without appropriate supplements to permit the counting of double auxotrophs and prototrophs. The intergenic recombination frequency is the summed values of double prototrophs and double auxotrophs as a percentage of viable spores.

Recombination frequencies were used to determine the genetic distance (cM) by employing Haldane's mapping function (genetic distance [cM] $= -50\ln[1-2R]$, where $R$ = the total fraction of recombinant spores amongst all spores analysed) [53].

### 2.6. Whole cell protein extraction and Western blots

Whole cell protein extracts (WCEs) were obtained following the protocol described by Ilyushik et al. [54] with the addition of a specific protease inhibitor set (Roche Molecular Biochemicals, Lewes, UK). The protein concentrations were determined and roughly 30 μg of WCE was run on a 10% polyacrylamide SDS-PAGE gel. The protein was then electroblotted onto PVDF transfer membrane (Amersham Biosciences UK Limited, Little Chalfont, UK). The blot was probed using anti-Translin and anti-TRAX polyclonal antibodies (see above). Donkey anti-rabbit IgG-HRP (Santa Cruz Biotechnology), and donkey anti-guinea pig IgG-HRP (Jackson Immuno Research), were used as secondary antibodies for Translin and Trax blots respectively. The blots were also probed using monoclonal anti-tubulin antibody (Sigma-Aldrich [T5168]) and goat anti-mouse IgG-HRP secondary antibody (Santa Cruz Biotechnology). ECL chemiluminescence technique was used as described by the manufacturer (Roche) to visualize the blot.

### 2.7. RNA extraction and Northern blotting

Cells were harvested and washed in 1 ml pre-chilled DEPC-treated water. Cells were harvested and resuspended in 750 μl TES buffer (10 mM Tris [pH 7.5], 10 mM EDTA [pH 8.0], 0.5% SDS), 750 μl of acidic phenol chloroform (Sigma) was added immediately, vortexed and incubated at 65 °C for 1 h. Samples were then placed on ice for 1 min followed by vortexing and centrifugation at 14,000 r.p.m. at 4 °C. Aqueous phase was removed, mixed with a fresh 700 μl of acidic phenol chloroform and recentrifuged. The aqueous phase was removed and mixed in a fresh Eppendorf tube with 1.5 ml pre-chilled (−20 °C) 100% ethanol and 50 μl of 3 M NaAc (pH 5.2). Samples were incubated at −20 °C overnight. RNA was harvested by centrifugation at 14,000 r.p.m. at room temperature. Supernatant was discarded and the pellet washed once with cold (4 °C) 70% ethanol. The pellet was air dried for 5 min. The pellet was finally resuspended in DEPC-treated $dH_2O$ and stored at −20 °C until required.

RNA electrophoresis and Northern blotting was carried out as described by Ilyushik et al. [54].

## 3. Results

### 3.1. Regulation of Trax expression by translin is functionally conserved in lower eukaryotes

Homologues of both translin and TRAX have been identified in *S. pombe* [34] (http://www.genedb.org/genedb/pombe/index.jsp; in this report we shall refer to *S. pombe* translin as *tsn*$^+$ and *S. pombe* TRAX as *trax*$^+$). Previously, it has been reported that levels of TRAX in MEFs, and *Drosophila* cells, are dependent upon translin [22,37,38]. Establishing whether this biochemical function for translin is conserved in a unicellular lower eukaryote will contribute to determining whether this activity is tissue-specific, or whether it serves some more fundamental role. To address this we raised polyclonal antibodies against Trax and Tsn proteins (see Methods). Consistent with previous analyses [34], we found that Tsn- and Trax-specific bands migrated with mobility of approximately 23 kDa in a 10% SDS-PAGE gel, which indicates both proteins migrate faster than the

predicted protein size (27.3 kDa for *S. pombe* Tsn and 26.7 kDa for *S. pombe* Trax; http://www.genedb.org/genedb/pombe/index.jsp; Fig. 1 A and B). We used these antibodies to measure the stability of the Trax in the *tsnΔ* strain (see below and Methods for a description of the generation of the null mutants). Fig. 1C shows that Trax cannot be detected in *tsnΔ* cells at moderate chemiluminescent exposure of a Western blot probed with anti-Trax antibodies, demonstrating that the translin function required for the stable expression of TRAX is conserved in the lower eukaryote *S. pombe*. However, on prolonged exposure, a Trax-specific band is observed, indicating that low levels of Trax are present in the *tsnΔ* mutant (Fig. 1D). This species is not detectable in a prolonged exposure of a Western blot of WCEs from the *traxΔ* mutant (Fig. 1D). Whilst a reduction of Trax in translin-deficient cells has been reported for MEFs and *Drosophila* cells, the presence of residual Trax has not been widely observed [22,37,38]. Furthermore, the over exposure revealed three Trax-specific species which migrate with molecular weights in the approximate range of 30–48 kDa (Fig. 1D; black arrows), suggesting that some cellular Trax exists in modified forms (see below).

The regulation of TRAX protein levels has been shown to be post transcriptional in nature in higher eukaryotes [37]; consistent with this we find no change in *trax*$^+$ mRNA levels in the *S. pombe tsnΔ* strain, indicating the post transcriptional regulation is also apparent in a unicellular lower eukaryote (Fig. 1E).

Trax protein levels are restored to TB-RBP (mouse translin)-deficient MEFs when they are treated with the mammalian proteasome inhibitor MG-132 [37]. This indicates that Trax is regulated by ubiquitin-mediated proteolysis and in support of this ubiquitinated Trax has been identified in MEFs [37]. Over exposure of WCE Western blots from wild-type *S. pombe* cells probed with anti-Trax antibodies revealed a ladder of higher molecular weight Trax-specific species (Fig. 1D); whilst the molecular weight shift between these species was less than that expected for ubiquitin monomers, 8.4 kDa, it remains a formal possibility that at least one of these species is a ubiquitinated form of Trax and that inhibition of the proteasome in cells with no translin (*tsnΔ*) might restore levels of Trax in the same fashion observed for MG-132 treated MEFs. To test this we generated a *tsnΔ* mutant which also carried a temperature sensitive allele of the gene coding for the Mts3 protein, which is an essential component of the *S. pombe* proteasome [55]. Whilst no Trax was detectable in the *tsnΔ* strain at the *mts3-1* non-permissive temperature (37 °C), Trax was detectable in the *tsnΔ mts3-1* double mutant at this temperature (Fig. 2). Consistent with this there was a slight elevation in Trax levels in the *mts3-1* single mutant at the restrictive temperature (Fig. 2). However, the levels of Trax protein in a *tsnΔ* background are not restored to *tsn*$^+$ levels by the inhibition of proteasome activity, indicating that another Translin-dependent, proteasome-independent pathway is controlling levels of Trax.

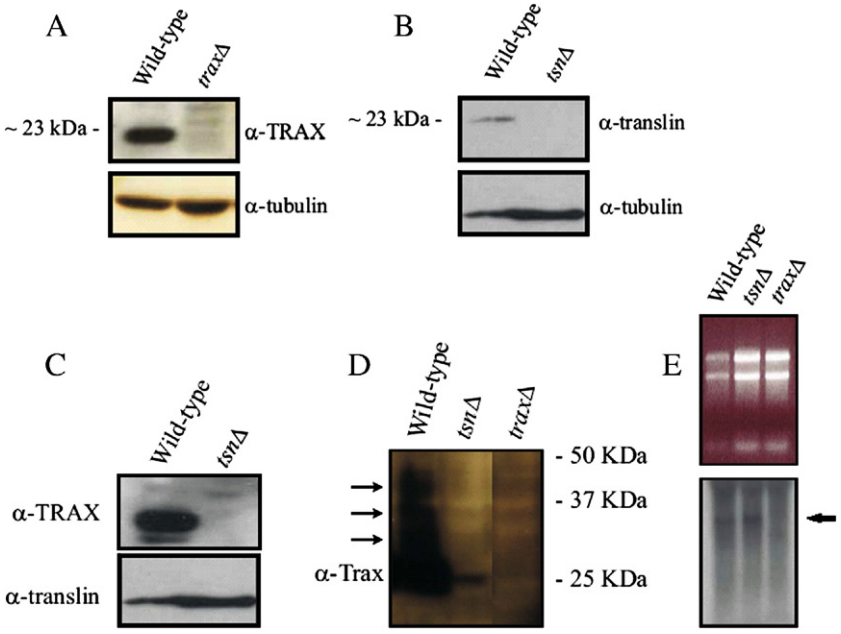


Fig. 1. Trax is depleted in Tsn-deficient cells. A and B. Whole cell protein extracts (WCEs) were made from wild-type, *traxΔ* (A) and *tsnΔ* strains. These were subjected to SDS-PAGE and Western blot analysis with polyclonal antibodies raised against Trax peptides (A) or Tsn peptides (B). Both sets of antibodies give a specific band at approximately 23 kDa. C. WCEs were made from wild-type and *tsnΔ* mitotically proliferating cells were subjected to SDS-PAGE and Western blotting. Anti-Trax antibodies were used to probe the blots and whilst a strong Trax-specific signal was detected with a mobility corresponding to approximately 23 kDa in the wild-type (left hand lane), no Trax-specific signal could be detected in the *tsnΔ* strain WCE following moderate exposure. D. Greatly prolonged exposure results in the detection of a Trax-specific band in the *tsnΔ* mutant which is not apparent in the *traxΔ* mutant. Prolonged exposure also reveals at least three Trax-specific bands which migrate with higher molecular weights in the wild-type (black arrows), indicating modified forms of Trax exist in mitotically proliferating wild-type cells. E. *trax*$^+$ mRNA levels are not altered in a *tsnΔ* strain, indicating that Tsn-dependent regulation of Trax stability is post transcriptional in nature. The lower panel is a Northern blot using a *trax*$^+$-specific probe; it shows a weak single *trax*$^+$-specific band with similar intensity in both the wild-type and the *tsnΔ* strain; this band is lost in the *traxΔ* strain. The upper panel is total RNA stained with ethidium bromide indicating uniform total RNA loading.

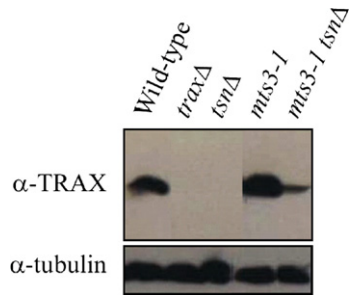


Fig. 2. Loss of proteasome function results in restoration of some, but not all Trax in translin-deficient cells. Stable expression of Trax protein is greatly reduced in the *tsnΔ* mutant (Fig. 1). The Western blot shows levels of Trax protein are elevated in both *tsn*$^+$ and *tsnΔ* backgrounds when the Mts3 proteasome subunit is inactivated by shifting the *mts3-1* temperature sensitive mutant to the restrictive temperature (37 °C). All WCEs are from exponentially growing cells shifted to 37 °C for 4 h.

### 3.2. Tsn and Trax do not play a primary role in regulating cell proliferation

Translin-deficient MEFs have a slow growth phenotype, suggesting that translin functions in controlling mitotic cell proliferation in metazoans [37]. To explore whether this was the case in lower eukaryotes we generated deletion mutants of both the translin (*tsn*$^+$) and TRAX (*trax*$^+$) genes in haploid *h*$^-$ *S. pombe* cells (see Methods). As with other deletion mutants previously generated [34] we found null mutants of both *tsn*$^+$ and *trax*$^+$ to be viable, with cell proliferation rates similar to the wild-type at a range of temperatures (20 °C, 25 °C, 30 °C, 33 °C and 37 °C) and no differences in cell morphology or size were noted between mutants and wild-type (data not shown). Both mutants appeared to recover from stationary phase with similar kinetics to the wild-type and mating and meiosis also appeared to proceed normally, with timing and asci morphology being indistinguishable from the wild-type for both *tsnΔ* and *traxΔ* strains (data not shown).

Previous reports have found that over expression of translin accelerates cell proliferation in higher eukaryotes and that depletion of translin and TRAX reduces cell proliferation [37,41,42]. However, loss of *S. pombe* translin and Trax appears to have little overt effect on rates of cell proliferation (see above). To investigate whether over expression would alter proliferation kinetics in a simple eukaryote we cloned the *S. pombe tsn*$^+$ and *trax*$^+$ genes under control of the inducible, high expression promoter, *nmt* (see Methods). Elevated expression of *tsn*$^+$ did not significantly alter the cell proliferation rate over a 10 h period of logarithmic growth following growth in the absence of thiamine for a period of 16 h (Fig. 3 A and C). When the plasmid pSRSB3 was induced to over express *trax*$^+$ (in the absence of thiamine) Northern blotting revealed a large increase in expression of the *trax*$^+$ mRNA (Fig. 3D); however, no corresponding increase was observed in the Trax protein levels (Fig. 3E) and no change in growth rate was observed (data not shown). Two prominent mRNA bands of approximately 1.0 and 1.5 kbs were observed to hybridise with a *trax*$^+$-specific probe when *trax*$^+$ is over expressed (Fig. 3D), in keeping with previous observations for murine and human TRAX mRNA [56,57]. However, when endogenous *trax*$^+$ mRNA levels were analysed by Northern blot only a single unique mRNA species was observed at relatively low levels (high exposures are required to identify this band; Fig. 1E). The lack of elevated Trax protein, when the *trax*$^+$ mRNA is greatly elevated, indicates that there is a post transcriptional regulatory pathway controlling the levels Trax expression in *S. pombe*. Given that Tsn is required for regulation of Trax levels (Fig. 1), we explored the possibility that elevated Tsn was required for over expression of Trax. We co-transformed wild-type cells with plasmids over expressing *trax*$^+$ (pSRSB3) and *tsn*$^+$ (pAJ1). These strains exhibited no change in growth rate (Fig. 3B). However, despite Tsn being over expressed, no measurable over expression of Trax could be detected in these cells (Fig. 3F); this suggests that over expression of Tsn does not result in the stimulation of higher levels of Trax protein when mRNA containing the *trax*$^+$ orf is expressed at high levels.

### 3.3. tsnΔ and traxΔ mutants exhibit no overt defect in response to a range of DNA damaging agents, salt or TBZ

Previous works in higher eukaryotes have, to some degree, implicated both TRAX and translin in the response to DNA damage (see Introduction). However, no direct evidence exists to indicate that either protein is required for cell recovery from DNA damage, although previous studies have explored only limited DNA damage response pathways. We took advantage of the fact that subtle sensitivities to DNA damaging agents can be readily tested using *S. pombe*. We assayed the sensitivity of the *tsnΔ* and *traxΔ* mutants to an extensive range of DNA damaging agents [ultra violet irradiation, mitomycin C (MMC), camptothecin, methyl methane sulfonate (MMS), phleomycin, cisplatin] and the DNA replication inhibitor hydroxyurea (HU); this enables us to explore a range of DNA damage recovery pathways, which have previously gone untested or uncorroborated. Sensitivity was tested at a range of temperatures [20 °C, 30 °C and 37 °C]. No sensitivity to any of these agents was observed at any temperature tested. Fig. 4C shows an example of the sensitivity test for mitomycin C and cisplatin for *tsnΔ* at 30 °C.

To date no studies have explored the possibility that these proteins have a role in a wider response to cellular stress. To explore this we determined whether or not *tsn*$^+$ and/or *trax*$^+$ have a role in the stress response and/or osmotic stress response pathways. We tested the *tsnΔ* and *traxΔ* strains for sensitivity to salt (10 mM KCl) and sorbitol (1.5 M). Neither strain was sensitive at any temperature tested (20 °C, 30 °C and 37 °C) (see Fig. 4A, for example).

In higher eukaryotes translin localises to centrosomes and microtubles, possibly suggesting a role in regulating the spindle apparatus [17,42,58]. Many *S. pombe* mutants defective in spindle and kinetochore function are sensitive to the microtubule depolymerising drug thiabendazole (TBZ). To test whether Tsn and Trax have a direct role to play in regulation of the spindle apparatus we tested *tsnΔ* and *traxΔ* cells for sensitivity to TBZ. However, we detected no sensitivity to this agent for either mutant (for example, see Fig. 4B).

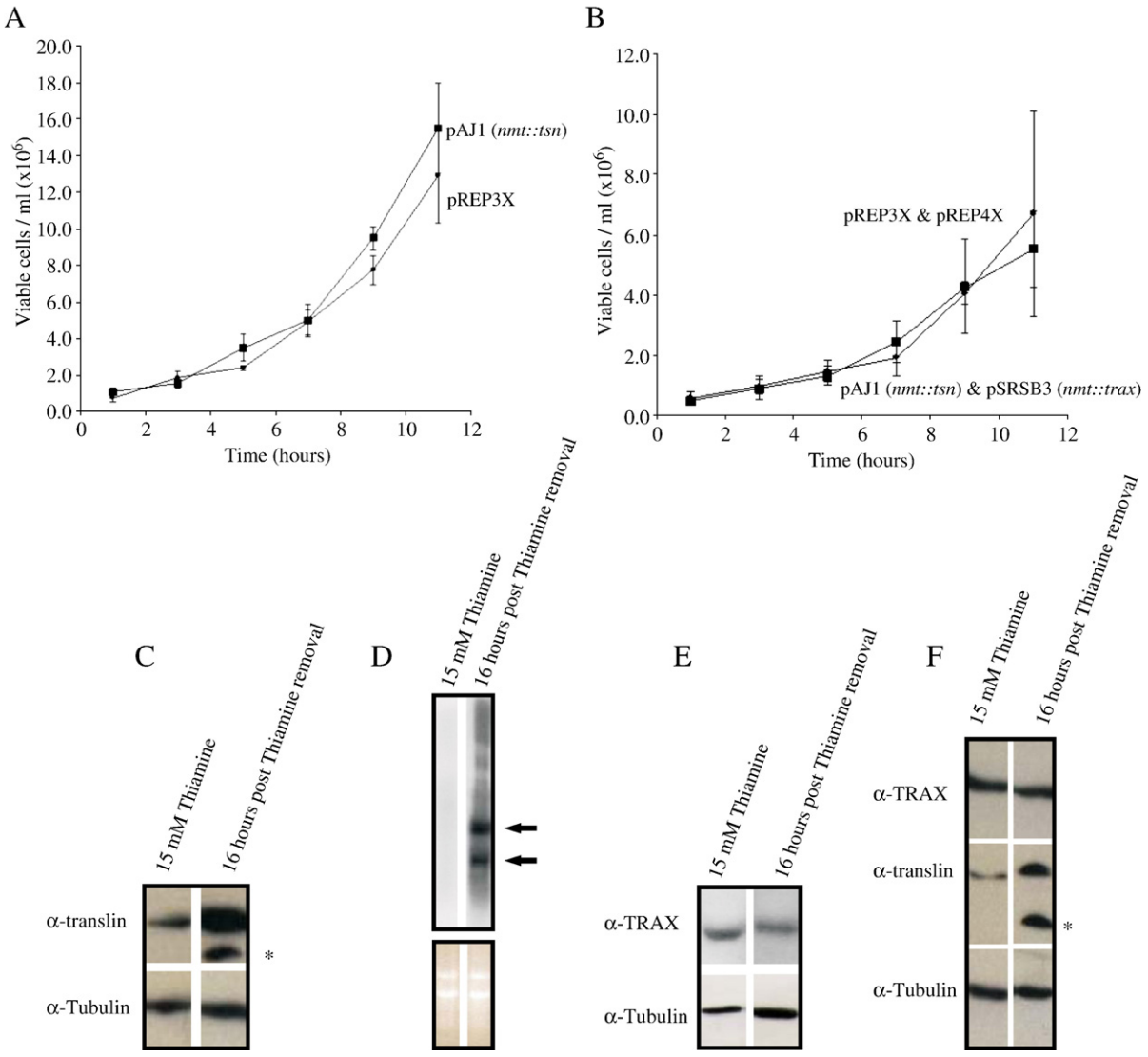


Fig. 3. Over expression of *tsn*$^+$ and *trax*$^+$. A. *tsn*$^+$ was over expressed on the plasmid pAJ1 under the regulation of the *nmt* promoter, which is induced after 16 h in the absence of thiamine. No difference could be detected in growth rate between pAJ1 (*tsn*$^+$) and the vector control (pREP3X). No measurable difference was noted in cell size or morphology (data not shown). Western blot analysis was carried out to demonstrate that the Tsn protein was over expressed from pAJ1 in the absence of thiamine (C; asterisk indicates a Tsn-related band which we believe to be a Tsn degradation product detectable in some WCEs). B. Plasmids with *tsn*$^+$ (pAJ1) and *trax*$^+$ (pSRSB3) under the regulation of the *nmt* promoter were transformed into a wild-type strain (both plasmids have different selectable markers). Under expression conditions (no thiamine) no major difference in growth rate was observed relative to the control strain containing empty vectors (pREP3X and pREP4X). No difference in cell size or morphology was noted (data not shown). Western blot analysis of WCEs from strains carrying both plasmids with regulatable *tsn*$^+$ (pAJ1) and *trax*$^+$ (pSRSB3) show that whilst Tsn protein is over expressed Trax is not (F). *trax*$^+$ expression under the regulation of the *nmt* promoter did not result in any measurable over expression of the Trax protein following 24 h in the absence of thiamine (E) when in the absence of the *tsn*$^+$ over expression (pSRSB3 alone); however, the levels of *trax*$^+$-specific mRNA species increases dramatically in this period without thiamine (D), indicating the *nmt* promoter is being induced at the transcriptional level (lower panel is total RNA stained with ethidium bromide, showing equal loading of total RNA). Two species of mRNAs predominate which migrate with an approximate size of 1.0 kb and 1.5 kp in cells over expressing *trax*$^+$ (black arrows). All growth data shown were from cultures grown at 30 °C.

### *3.4. The tsnΔ mutant exhibits no defect in non-homologous DNA end joining (NHEJ)*

Due to the possible role of translin in generating chromosomal translocations, it has been suggested that translin might function in NHEJ. *S. pombe* mutants defective in NHEJ, such as *lig4Δ*, are similar to *tsnΔ* and *traxΔ* strains as they exhibit no measurable sensitivity to DNA damaging agents [59]. The proposal that suggests translin functions in a NHEJ pathway has not previously been formally tested. To do so we employed a NHEJ plasmid recircularisation assay. In brief, plasmids are linearised with restriction endonucleases which generate 5′, 3′ or blunt termini; these linear plasmids are then transformed into cells and the transformation frequency is used as a metric for the efficiency of plasmid recircularisation. Given that the plasmid (pFY20 in this case; 48) has no extensive internal homology, then recircularisation is either by perfect religation or a non-homologous recombination reaction, resulting in deletion of some plasmid DNA (both are referred to as NHEJ). A value for NHEJ efficiency is represented by an *L*/*C* ratio which is generated by dividing the transformation frequency obtained with linear plasmid DNA (*L*) with that obtained with covalently

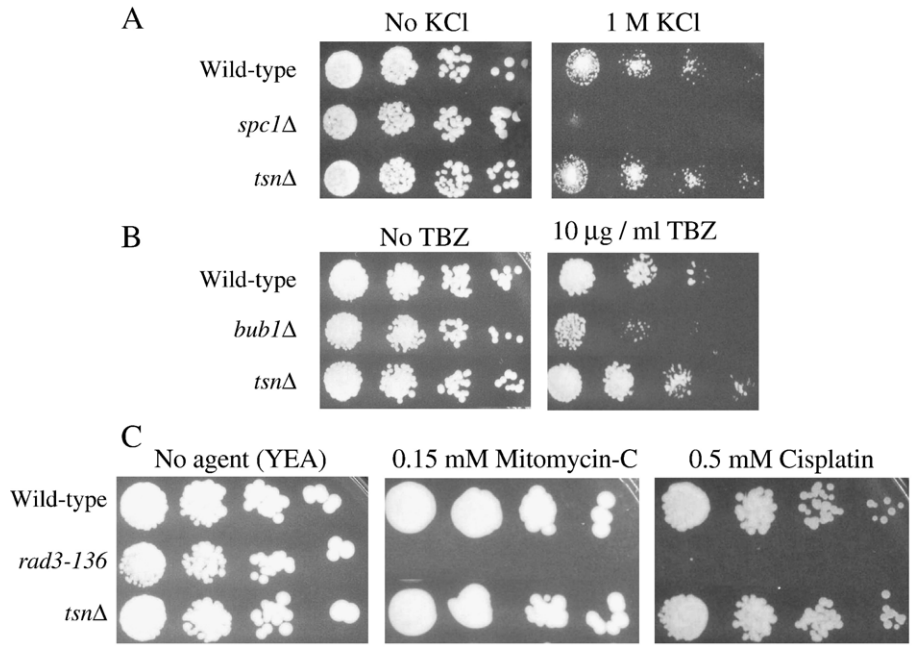


Fig. 4. Example of robust response to cellular insult by *tsnΔ* cells. A. *tsnΔ* cells exhibit no defect in salt (1 M KCl). *spc1Δ* cells, which are highly sensitive to 1 M KCl, are shown as a control. B. *tsnΔ* cells exhibit no sensitivity to the microtubule depolymerising agent thiabendazole (TBZ; 10 µg/ml). The *bub1Δ* strain exhibits sensitivity to this agent and this strain is used as a positive control. C. Examples of the lack of sensitivity of the *tsnΔ* strain to DNA damaging agents (cisplatin and mitomycin C); *rad3-136* cells are used as a positive control. All images were from plates incubated at 30 °C and all show serial dilutions of log phase cultures ($10^{-2}$ to $10^{-5}$, from left to right).

closed circular DNA (*C*) (uncut pFY20), which normalises for any strain to strain variance in transformation efficiency (rather than NHEJ efficiency). Table 2 shows that whilst the *lig4Δ* strain (known to be defective in NHEJ; [59]) has greatly impaired NHEJ, the *tsnΔ* strain was similar to the wild-type, irrespective of the structure of the linear DNA termini. Given the depletion of Trax in the *tsnΔ* strain (Fig. 1) the *traxΔ* strain was not tested.

### 3.5. The tsnΔ mutant exhibits no defect in mitotic or meiotic homologous recombination

It has been suggested that translin functions in regulating a mitotic recombination pathway, but this remains untested. To determine whether *S. pombe* Tsn has a primary function in mitotic recombination we employed a sensitive plasmid-by-chromosome recombination assay. In brief, cells carrying the *ade6-M26* mutant allele at the *ade6* chromosomal locus are transformed with a circular plasmid carrying a second *ade6* mutant allele, *ade6-469*; the *ade6-M26* and *ade6-469* alleles were generated by point mutations which are 1332 base pairs apart and so recombination between these alleles can generate conversion of either mutation to the $ade6^{+}$ sequence, which can be measured genetically as adenine prototrophy. Using this system, fluctuation analyses were carried out on wild-type, *traxΔ* and *tsnΔ* strains [51]. Fig. 5A shows that the frequency of adenine prototroph production was not significantly different between wild-type, *tsnΔ* and *traxΔ* strains.

Translin binding sites have been detected in male meiotic recombination hot spots, suggesting a possible role for the regulation of meiotic recombination [9]. Whilst crossing over (CO) levels have previously been tested in *Drosophila* and were normal for the intervals tested [38] a role for translin in regulating gene conversions (GCs), which can occur via mechanistically distinct

Table 2
Non-homologous DNA end joining of linear DNA with 3′, 5′ and blunt termini is not impaired in translin-deficient cells

| | *Pst*I (3′ over hang) | | *Sma*I (blunt ended) | | *Xma*I (5′ over hang) | |
|---|---|---|---|---|---|---|
| | Mean $L/C$ [a, b] | % of wild-type | Mean $L/C$ [a, b] | % of wild-type | Mean $L/C$ [a, b] | % of wild-type |
| Wild-type | 0.21 (0.20) | – | 0.37 (0.37) | – | $3.0\times10^{-3}$ ($1.0\times10^{-3}$) | – |
| *lig4Δ* [d] | $<3.1\times10^{-4}$ [c] ($3.0\times10^{-4}$) | <0.15 | $4.7\times10^{-4}$ ($4.7\times10^{-4}$) | 1.3 | $<2.2\times10^{-4}$ [c] ($3.0\times10^{-4}$) | <7.3 |
| *tsnΔ* | 0.2 (0.15) | 95.2 | 0.49 (0.27) | 132.4 | $6.0\times10^{-3}$ ($6.0\times10^{-3}$) | 200.0 |

[a] The *L*/*C* ratio is the ratio of the number of transformants per viable cell obtained using linearised pFY20 plasmid DNA to the number of transformants per viable cell obtained for covalently closed circle pFY20 plasmid DNA.

[b] $n=3$ in all cases. Standard deviation is given in parentheses. Pair wise comparison of wild-type and *tsnΔ* values using Student's *t*-test gave *P*-values > 0.05 in all cases.

[c] No transformants were obtained; the value of 3 was used as the number of transformants obtained for the calculation of the *L*/*C* ratio.

[d] This strain carries the *ade6-704* allele, whereas the wild-type and the *tsnΔ* strain carry the *ade6-M26* allele.

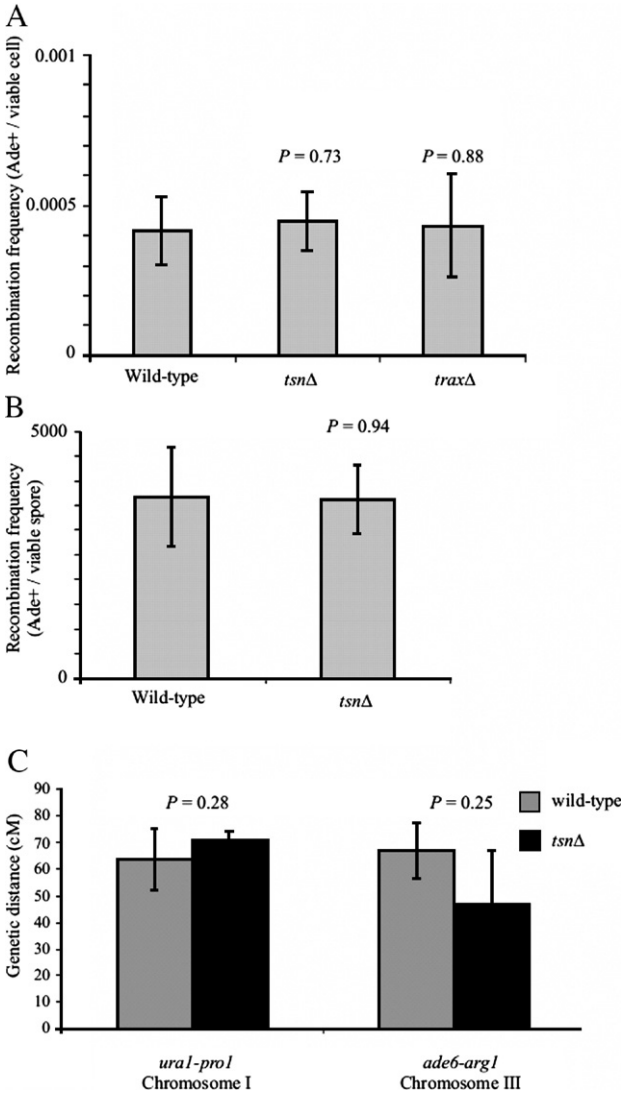


Fig. 5. *tsnΔ* cells are not defective in mitotic or meiotic recombination. A. Mitotic plasmid-by-chromosome recombination is not reduced in *tsnΔ* and *trxΔ* mutants. See the main text for a brief outline of the assay system. B. Meiotic intragenic recombination is not altered in the *tsnΔ* mutant. C. Intergenic meiotic recombination was measured at two intervals, *ura1–pro1* on chromosome I and *ade6–arg1* on chromosome III, in both wild-type and *tsnΔ* strains. Genetic distances were calculated by use of Haldane's mapping function as described in [for example, see 53]. In all cases (A–C) error bars show standard deviations and pair wise comparison of wild-type and *tsnΔ* genetic distances using Student's *t*-test give *P*-values which are shown within the plot.

pathways [60,61], has not been tested in any system. We tested meiotic genetic recombination, both intragenic (GC), at the *ade6* locus, and intergenic (CO), at two separate intervals. Two factor crosses using the *ade6-M26* hot spot allele and the *ade6-52* marker allele show no differences between the wild-type and the *tsnΔ* strains (Fig. 5B); this shows there is no loss of meiotic intragenic recombination or *ade6-M26* hot spot activation.

Intergenic recombination, crossing over, was measured at the *pro1*$^+$*–ura1*$^+$ intervals on chromosome I and *ade6*$^+$*–arg1*$^+$ interval on chromosome III. These intervals were chosen to represent one locus for the different meiotic recombination regions in *S. pombe* [62], and because they are adjacent to different prominent meiotic double-strand break sites (*mbs1* for *pro1*$^+$*–ura1*$^+$; *ade6-M26* for *ade6*$^+$*–arg1*$^+$). Fig. 5C shows that genetic distance for these intervals are statistically indistinguishable for the *tsnΔ* mutant relative to the wild-type.

### 3.6. GT microsatellite repeats remain stable in tsnΔ and traxΔ strains

*S. pombe* translin binds selectively to single-stranded d[GT]$_n$ and d[GTT]$_n$ DNA repeats [34]. Alterations to the stability of microsatellite repeat sequences can result in human genetic disease, many associated with neurological disorders [for example, see 63] and cancer [for example, see 64]. Microsatellite repeat instability has been linked to impaired mismatch repair (MMR) systems and, in *S. pombe,* GT microsatellite repeat stability is dependent upon MMR pathways [65]. We employed the system of Mansour et al. [65] to study [GT]$_8$ repeat stability in Tsn- and Trax-deficient cells to determine whether the selective binding of Tsn to d[GT]$_n$ repeats [34] reflected a function in regulation of microsatellite repeat stability. This system is

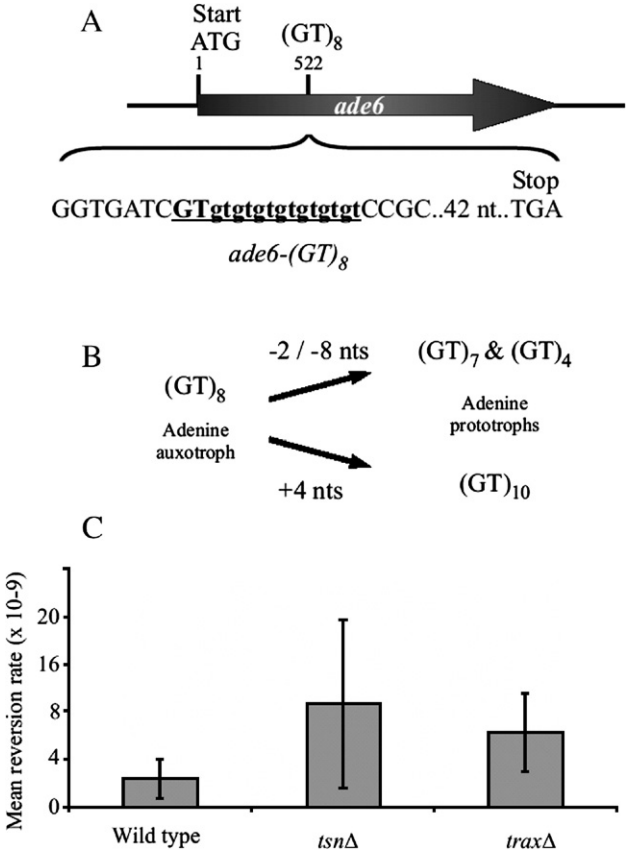


Fig. 6. *tsnΔ* cells exhibit a small increase in GT microsatellite repeat instability. A. [GT]$_8$ repeat is integrated at position 522 within the *ade6* open reading frame [65]. B. Frame shifts, due to loss or gain of GT repeats, can result in expression of a functional Ade6 protein capable of conferring adenine prototrophy; loss of one [GT] or four [GT]$_4$ repeats or gain of two [GT]$_2$ repeats results in adenine prototrophy. C. *tsnΔ* and *traxΔ* cells exhibit a small, but insignificant, increase in the rate of GT microsatellite instability (Student's *t*-test gives *P*-values > 0.05 in pair wise comparisons of mutants with wild-type). Fluctuation analyses were carried out and mean reversion rates are calculated from at least three independent median values obtained from independent experiments (error bars are standard deviations).

described in detail elsewhere [65], but, in brief, it consists of eight GT repeats inserted within the *ade6* open reading frame resulting in inactivation of the *ade6* gene (Fig. 6A); change in the number of GT repeats can result in an in-frame open reading frame for *ade6* which restores adenine prototrophy; this occurs with loss of one ($[GT]_7$; 2 nucleotides) or four ($[GT]_4$; 8 nucleotides) repeats or gain of two repeats ($[GT]_{10}$; 4 nucleotides) (Fig. 6B).

The rate of adenine prototrophy formation for *tsnΔ* and *tsn*$^+$ cells carrying $[GT]_8$ repeats was carried out by fluctuation analysis [51]. A small rise in $[GT]_8$ instability was observed for *tsnΔ* and *traxΔ* cells (Fig. 6C), but these are not statistically significant, although a broader range of values was observed for the mutants, particularly the *tsnΔ* strain, possibly indicating a subtle underlying effect.

## 4. Discussion

### 4.1. Translin function is conserved in lower eukaryotes

The high level of conservation of the TRAX and translin proteins seems to suggest that they play an important biological role which provides a selective advantage. In both mouse and *Drosophila* the translin protein is required for the stable expression of TRAX protein [22,37,38]. We have demonstrated that whilst amino acid sequence identity between *S. pombe* murine and *Drosophila* translin proteins is 35% and 31% respectively, there is functional conservation as *S. pombe* Tsn, like murine and *Drosophila* translin, is required for stable expression of the *S. pombe* Trax protein. It has been suggested that the regulation of TRAX levels by translin is by direct translin-TRAX interaction controlling the levels of ubiquitin-mediated TRAX proteolysis [37,38]. Our data indicate that such regulatory interaction is highly conserved. In MEFs chemical inhibition of the proteasome results in a full restoration of TRAX levels to levels seen in translin-proficient cells [37]. By mutating an *S. pombe* proteasome sub-unit we restore some Trax, consistent with the proposal that translin functions to protect Trax from proteasome degradation. However, we do not observe restoration of wild-type levels of Trax in the *S. pombe* proteasome mutant. This might be due to the fact that the *mts3-1* conditional mutant is functionally leaky and that residual proteasome activity is sufficient to mediate some Trax degradation in the absence of translin, possibly indicative of Trax being highly labile. Alternatively, we cannot dismiss a second role for translin in positively regulating the translation of *trax*$^+$ mRNA.

Whatever the function of translin in mediating Trax levels, our data indicate that this biological process is important for both unicellular eukaryotes and metazoans. This might suggest that the conserved function does not relate to tissue-specific biological processes in metazoans as it is conserved in unicellular eukaryotes.

In this study we over expressed the *trax*$^+$ mRNA. However, this did not result in a measurable increase in the Trax protein. This suggests that the levels of Trax protein in the cell are determined by post transcriptional regulation, consistent with the proposal that translin is required for efficient translation of the *trax*$^+$ mRNA (see above). If Tsn is responsible for Trax stabilisation, then this regulation is more complex as co-over expression of Tsn in the presence of higher levels of *trax*$^+$ mRNA did not increase the measurable Trax in the cell. It might be possible that we have failed to clone untranslated regions (UTRs) associated with the *trax*$^+$ orf which are essential for translation and that the control of Trax translation is regulated by as yet unidentified, UTR-dependent mechanisms, possibly requiring Tsn. The system we have developed here will provide a tool for further exploration of this possibility.

### 4.2. What is the biological function of Tsn and Trax?

In this report we have extensively explored the possibility that Trax and Tsn play a fundamental role in the maintenance of the genome integrity, meiotic/mitotic recombination and the cell growth process. We find no defects in any of these processes, other than a small increase in GT repeat instability. This can be explained in one of two ways; firstly, neither gene plays a prominent role in any biological pathway important to laboratory cultured cells; secondly, the pathways these proteins function in may be redundant and other pathways can substitute for Trax and Tsn function. It has been suggested that the neurological defects in mice and flies defective in translin [22–24], and the binding of translin to neuronal mRNAs [for example, see 13], implicates translin function in neuronal mRNA metabolism; moreover, it has been postulated that the conservation of function is important as loss of neural function would confer a serious selective disadvantage to flies and mammals. However, this does not account for conservation of sequence and function in the fission yeast. A simple explanation for this incongruity is that *S. pombe* Trax and Tsn may function in the regulation of other subsets of mRNAs and that higher eukaryotes have evolved a tissue specificity for this function. If *S. pombe* Trax and Tsn are involved in mRNA metabolism loss of function appears to result in little overt phenotype in laboratory cultured strains; however, it might be the case that they function in a condition-dependent fashion which has yet to be evaluated. The findings that *S. pombe* Tsn has a greater affinity for RNA repeat sequences than DNA supports a role in RNA metabolism [34].

We have extensively studied the sensitivity of both *tsnΔ* and *traxΔ* cells to DNA damaging agents and we find no indications that either protein is required for recovery from DNA damage, dispelling the suggestion that these proteins are evolutionarily conserved due to a fundamental role in the DNA damage response. In HeLa cells mild increases in nuclear translin were observed in response to the chemotherapeutic agents mitomycin C and cisplatin [25], but here we have demonstrated that in *S. pombe* *tsnΔ* and *traxΔ* cells are as resistant to both of these agents as wild-type cells, indicating that any increase in nuclear amount of Tsn is not essential for recovery from the DNA damage these agents generate. This is consistent with later findings that both MEFs and *Drosophila* embryos defective in translin exhibit no increase in sensitivity to some DNA damaging agents relative to their translin proficient isogenic controls [37,38]. Furthermore, it has been suggested that because TRAX interacts with C1D, a protein required for mitotic recombination, that TRAX might have a role

in the regulation of mitotic recombination [44,45]; however, we found no evidence to substantiate that proposal.

### 4.3. Do Trax and Tsn regulate GT repeat stability?

Whilst we observe no measurable loss of recombination function, we did note a small, increase in the instability of a $[GT]_8$ repeat sequence artificially introduced into the *ade6* locus in both mutants. Whilst this was not reproducibly statistically significant, this is largely due to the large ranges in values we obtained for the *tsnΔ* mutant, which exhibited a greater mean increase in instability than the *traxΔ* strain. This might reflect some heterogeneity within the population of *tsnΔ* cells with respect to the way GT repeat sequence stability is maintained. Further analysis into the nature of this broad range of GT instability in *tsnΔ* cells may yet uncover a role for these conserved proteins in genomic regulation.

### 4.4. Closing remarks

We have extensively analysed the phenotypes of *S. pombe* *tsnΔ* and *traxΔ* cells. We find no strong evidence to indicate that these proteins function in cell cycle regulation, recombination or DNA damage recovery. However, we do find functional conservation which suggests that these enigmatic proteins play a role in a biological process which is important for both multicellular and unicellular eukaryotes, suggesting it is of fundamental biological importance. The finding that TRAX and translin seem to regulate cell proliferation in higher eukaryotes, but not in *S. pombe*, where the biochemical function is conserved, indicates that there is not a clear correlation between the conserved biochemical function and regulation of cell proliferation, suggesting the two are not linked. Further analysis in this simple eukaryote will provide insight into the nature of this process.

## Acknowledgements

We would like to thank Oliver Fleck for providing GT repeat strains and advice on their use. A.J. was funded by a BBSRC Quota studentship and DWP was funded by the North West Cancer Research Fund.

## References

[1] P.D. Aplan, Causes of oncogenic chromosomal translocation, Trends Genet. 22 (2006) 46–55.
[2] D.M. Weinstock, C.A. Richardson, B. Elliott, M. Jasin, Modeling oncogenic translocations: Distinct roles for double-strand break repair pathways in translocation formation in mammalian cells, DNA Repair 5 (2006) 1065–1074.
[3] K. Aoki, K. Suzuki, T. Sugano, T. Tasaka, K. Nakahara, O. Kuge, A. Omori, M. Kasai, A novel gene, Translin, encodes a recombination hotspot binding protein associated with chromosomal translocation, Nat. Genet. 10 (1995) 167–174.
[4] M. Atlas, D. Head, F. Behm, E. Schmidt, N.H. Zeleznik-Le, B.A. Roe, D. Burian, P.H. Domer, Cloning and sequence analysis of four t[9;11] therapy-related leukaemia breakpoints, Leukemia 12 (1998) 1895–1902.
[5] H. Kanoe, T. Nakayama, T. Hosaka, H. Murakami, H. Yamamoto, Y. Nakashima, T. Tsuboyama, T. Nakamura, D. Ron, M.S. Sasaki, J. Toquchida, Characteristics of genomic breakpoints in TLS-CHOP translocation in the liposarcomas suggest the involvement of Translin and topoisomerase II in the process of translocation, Oncogene 18 (1999) 721–729.
[6] T. Hosaka, H. Kanoe, T. Nakayama, H. Murakami, H. Yamamoto, T. Nakamata, T. Tsubyama, M. Oka, M. Kasai, M.S. Sasaki, T. Nakamura, J. Toquchida, Translin binds to the sequences adjacent to the breakpoints of the TLS and CHOP genes in liposarcomas with translocation t[12;6], Oncogene 19 (2000) 5821–5825.
[7] S.S. Abeysinghe, N. Chuzhanova, M. Krawczak, E.V. Ball, D.N. Cooper, Translocation and gross deletion breakpoints in human inherited disease and cancer I: nucleotide composition and recombination-associated motifs, Hum. Mutat. 22 (2003) 229–244.
[8] Y. Wei, M. Sun, G. Nilsson, T. Dwight, Y. Xie, J. Wang, Y. Hou, O. Larsson, X. Zhu, Characteristic sequence motifs located at the genomic breakpoints of the translocation t[X;18] in synovial sarcomas, Oncogene 22 (2003) 2215–2222.
[9] R.M. Badge, J. Yardley, A.J. Jeffreys, J.A. Armour, Crossover breakpoint mapping identifies a subtelomeric hotspot for male meiotic recombination, Hum. Mol. Genet. 9 (2000) 1239–1244.
[10] R. Visser, O. Shimokawa, N. Harada, A. Kinoshita, T. Ohta, N. Niikawa, N. Matsumoto, Identification of a 3.0-kb major recombination hotspot in patients with Soto syndrome who carry a common 1.9-Mb microdeletion, Am. J. Hum. Genet. 76 (2005) 52–67.
[11] M. Gajecka, C.D. Glotzbach, L.G. Shaffer, Characterization of a complex rearrangement with interstitial deletions and inversion on human chromosome 1, Chromosome Res. 14 (2006) 277–282.
[12] M. Gajecka, A. Pavlicek, C.D. Glotzbach, B.C. Ballif, M. Jarmuz, J. Jurka, L.G. Shaffer, Identification of sequence motifs at the breakpoint junctions in three t[1;9][p36.3;q34] and delineation of mechanisms involved in generating balanced translocations, Hum. Genet. 120 (2006) 519–526.
[13] X.Q. Wu, W. Gu, X. Meng, N.B. Hecht, The RNA-binding protein, TB-RBP, is the mouse homologue of translin, a recombination protein associated with chromosomal translocations, Proc. Natl. Acad. Sci. U. S. A. 94 (1997) 5640–5645.
[14] X.Q. Wu, P. Petrusz, N.B. Hecht, Testis–brain RNA-binding protein [Translin] is primarily expressed in neurons of the mouse brain, Brain Res. 819 (1999) 174–178.
[15] W. Gu, X.Q. Wu, X.H. Meng, C. Morales, M. El-Halfy, N.B. Hecht, The RNA- and DNA-binding protein TB-RBP is spatially and developmentally regulated during spermatogenesis, Mol. Reprod. Dev. 49 (1998) 219–228.
[16] T. Muramatsu, A. Ohmae, K. Anzai, BC1 RNA protein particles in mouse brain contain two y-, h-element-binding proteins, translin and a 37 kDa protein, Biochem. Biophys. Res. Commun. 247 (1998) 7–11.
[17] X.Q. Wu, N.B. Hecht, Mouse testis brain ribonucleic acid-binding protein/translin colocalizes with microtubules and is immunoprecipitated with messenger ribonucleic acids encoding myelin basic protein, alpha calmodulin kinase II, and protamines 1 and 2, Biol. Reprod. 62 (2000) 720–725.
[18] V. Chennathukuzhi, C.R. Morales, M. El-Alfy, N.B. Hecht, The kinesin KIF17b and RNA-binding protein TB-RBP transport specific cAMP-responsive element modulator-regulated mRNAs in male germ cells, Proc. Natl. Acad. Sci. U.S.A 100 (2003) 15566–15571.
[19] J. Yang, V. Chennathukuzhi, K. Miki, D.A. O'Brien, N.B. Hecht, Mouse testis brain RNA-binding protei/translin selectively binds to the messenger RNA of the fibrous sheath protein glyceraldehydes 3-phosphate dehydrogenase-S and suppresses its translation in vitro, Biol. Reprod. 68 (2003) 853–859.
[20] Z. Li, J.M. Baraban, High affinity binding of the Translin/Trax complex to RNA does not require the presence of Y or H elements, Brain Res. Mol. Brain Res. 120 (2004) 123–129.
[21] Y.S. Cho, N. Iguchi, J. Yang, M.A. Handle, N.B. Hecht, Meiotic messenger RNA and noncoding RNA targets of the RNA-binding protein Translin [TSN] in mouse testis, Biol. Reprod. 73 (2005) 840–847.
[22] V. Chennathukuzhi, J.M. Stein, T. Abel, S. Donlon, S. Yang, J.P. Miller, A.D. Allman, R.A. Simmons, N.B. Hecht, Mice deficient for testis–brain RNA-binding protein exhibit a coordinate loss of TRAX,

reduced fertility, altered gene expression in the brain, and behavioral changes, Mol. Cell. Biol. 23 (2003) 6419–6434.
[23] J.M. Stein, W. Bergman, Y. Fang, L. Davison, C. Brensinger, M.B. Robinson, N.B. Hecht, T. Abel, Behavioral and neurochemical alterations in mice lacking the RNA-binding protein translin, J. Neurosci. 26 (2006) 2184–2196.
[24] K. Suseendranathan, K. Sengupta, R. Rikhy, J.S. D'Souza, M. Kokkanti, M.G. Kulkarni, R. Kamdar, R. Changede, R. Sinha, L. Subramanian, K. Singh, V. Rodrigues, B.J. Rao, Expression pattern of *Drosophila* translin and behavioural analyses of the mutant, Eur. J. Cell Biol. 86 (2007) 173–186.
[25] M. Kasai, T. Matsuzaki, K. Katayanagi, A. Omori, R.T. Maziarz, J.L. Strominger, K. Aoki, K. Suzuki, The translin ring specifically recognizes DNA ends at recombination hot spots in the human genome, J. Biol. Chem. 272 (1997) 11402–11407.
[26] X.Q. Wu, L. Xu, N.B. Hecht, Dimerization of the testis brain RNA-binding protein [translin] is mediated through its C-terminus and is required for DNA- and RNA-binding, Nucleic Acids Res. 26 (1998) 1675–1680.
[27] K. Aoki, K. Suzuki, R. Ishida, M. Kasai, The DNA binding activity of Translin is mediated by a basic region in the ring-shaped structure conserved in evolution, FEBS Lett. 443 (1999) 363–366.
[28] K. Sengupta, B.J. Rao, Translin binding to DNA: recruitment through DNA ends and consequent conformational transitions, Biochemistry 41 (2002) 15315–15326.
[29] G.D. Gupta, R.D. Makde, R.P. Kamdar, J.S. D'Souza, M.G. Kulkarni, V. Kumar, B.J. Roa, Co-expressed recombinant human Translin–Trax complex binds DNA, FEBS Lett. 579 (2005) 3141–3146.
[30] M.S. Van Loock, X. Yu, M. Kasai, E.H. Egelman, Electron microscopic studies of the translin octameric ring, J. Struct. Biol. 135 (2001) 58–66.
[31] M.M. Hingorani, M. O'Donnell, A tale of toroids in DNA metabolism, Nat. Rev. Mol. Cell Biol. 1 (2000) 22–30.
[32] A. Aharoni, N. Baran, H. Manor, Characterization of a multisubunit human protein which selectively binds single stranded d[GA]$_n$ and d[GT]$_n$ sequence repeats in DNA, Nucleic Acids Res. 21 (1993) 5221–5228.
[33] E. Jacob, L. Pucshansky, E. Zeruya, N. Baran, H. Manor, The human protein translin specifically binds single-stranded microsatellite repeats, d[GT]$_n$, and G-strand telomeric repeats, d[TTAGGG]$_n$: a study of the binding parameters, J. Mol. Biol. 344 (2004) 939–950.
[34] O. Laufman, R. Ben Yosef, N. Adir, H. Manor, Cloning and characterisation of the *Schizosaccharomyces pombe* homologs of the human protein Translin and Translin-associated protein TRAX, Nucleic Acids Res. 33 (2005) 4128–4139.
[35] J. Wang, E.S. Boja, H. Oubrahim, P.B. Chock, Testis brain ribonucleic acid-binding protein/translin possesses both single-strand and double-strand ribonuclease activities, Biochemistry 43 (2004) 13424–13431.
[36] K. Aoki, R. Ishida, M. Kasai, Isolation and characterisation of a cDNA encoding a Translin-like protein, TRAX, FEBS Lett. 401 (1997) 109–112.
[37] S. Yang, Y.S. Cho, V.M. Chennathukuzhi, L.A. Underkoffler, K. Loomes, N.B. Hecht, Translin-associated factor X is post-transcriptionally regulated by its partner protein TB-RBP, and both are essential for normal cell proliferation, J. Biol. Chem. 279 (2004) 12605–12614.
[38] M. Clauβen, R. Koch, Z.Y. Jin, B. Suter, Functional characterisation of *Drosophila* Translin and Trax, Genetics 174 (2006) 1337–1347.
[39] Y.S. Cho, V.M. Chennathukuzhi, M.A. Handle, J. Eppig, N.B. Hecht, The relative levels of translin-associated factor X [TRAX] and testis brain RNA-binding protein determine their nucleocytoplasmic distribution in male germ cells, J. Biol. Chem. 279 (2004) 31514–31523.
[40] V.M. Chennathukuzhi, Y. Kurihara, J.D. Bray, N.B. Hecht, Trax [translin-associated factor X], a primarily cytoplasmic protein, inhibits the binding of TB-RBP [translin] to RNA, J. Biol. Chem. 276 (2001) 13256–13263.
[41] S. Yang, N.B. Hecht, Translin associated protein X is essential for cellular proliferation, FEBS Lett. 576 (2004) 221–225.
[42] R. Ishida, H. Okado, H. Sato, C. Shionoiri, K. Aoki, M. Kasai, A role for the octameric ring protein, translin, in mitotic cell division, FEBS Lett. 525 (2002) 105–110.
[43] T. Hasegawa, K. Isobe, Evidence for the interaction between Translin and GADD34 in mammalian cells, Biochem. Biophy. Acta 1428 (1999) 161–168.
[44] T. Erdemir, B. Bilican, D. Oncel, C.R. Goding, U. Yavuzer, DNA damage-dependent interaction of the nuclear matrix protein C1D with Translin-associated factor X [TRAX], J. Cell Sci. 115 (2002) 207–216.
[45] T. Erdemir, B. Bilican, T. Cagatay, C.R. Goding, U. Yavuzer, *Saccharomyces cerevisiae* C1D is implicated in both non-homologous DNA end joining and homologous recombination, Mol. Microbiol. 46 (2002) 947–957.
[46] S. Moreno, A. Klar, P. Nurse, Molecular genetic analysis of fission yeast *Schizosaccharomyces pombe*, Methods Enzymol. 194 (1991) 795–823.
[47] P. Szankasi, W.D. Heyer, P. Schuchert, J. Kohli, DNA sequence analysis of the *ade6* gene of *Schizosaccharomyces pombe*: wild type and mutant alleles including the recombination hot spot allele *ade6-M26*, J. Mol. Biol. 204 (1988) 917–925.
[48] Y.F. Li, M. Numata, W.P. Wahls, G.R. Smith, Region-specific meiotic recombination in *Schizosaccharomyces pombe*: the rec11 gene, Mol. Microbiol. 23 (1997) 869–878.
[49] K. Maundrell, Thiamine-repressible expression vectors pREP and pRIP for fission yeast, Gene 123 (1993) 127–130.
[50] J. Bähler, J.Q. Wu, M.S. Longtine, N.G. Shah, A. McKenzie III, A.B. Steever, A. Wach, P. Philippsen, J.R. Pringle, Heterologous modules for efficient and versatile PCR-based gene targeting in *Schizosaccharomyces pombe*, Yeast 14 (1998) 943–951.
[51] D.E. Lea, C.A. Coulson, The distribution of the number of mutants in bacterial populations, J. Genet. 49 (1949) 264–285.
[52] D.W. Pryce, A. Lorenz, J.B. Smirnova, J. Loidl, R.J. McFarlane, Differential activation of *M26*-containing meiotic recombination hot spots in *Schizosacchomyces pombe*, Genetics 170 (2005) 95–106.
[53] J.L. Wells, D.W. Pryce, A. Estreicher, J. Loidl, R.J. McFarlane, Linear element-independent meiotic recombination in *Schizosaccharomyces pombe*, Genetics 174 (2006) 1105–1114.
[54] E. Ilyushik, D.W. Pryce, D. Walerych, T. Riddell, J.A. Wakeman, C.J. McInerny, R.J. McFarlane, Psc3 cohesin of *Schizosaccharomyces pombe*: cell cycle analysis and identification of three distinct isoforms, Biol. Chem. 386 (2005) 613–621.
[55] C. Gordon, G. McGurk, M. Wallace, N.D. Hastie, A conditional lethal mutant in the fission yeast 26 S Protease subunit mts3+ is defective in metaphase to anaphase transition, J. Biol. Chem. 271 (1996) 5704–5711.
[56] R.S. Devon, M.S. Taylor, J.K. Millar, D.J. Poteous, Isolation and characterization of the mouse translin-associated protein X [Trax] gene, Mamm. Genome 11 (2000) 395–398.
[57] K.S. Millar, S. Christie, C.A.M. Semple, D.J. Porteous, Chromosomal location and genomic structure of the human translin-associated factor X gene [TRAX; TSNAX] revealed by intergenic splicing to DISC1, a gene disrupted by a translocation segregating with schizophrenia, Genomics 67 (2000) 69–77.
[58] A. Castro, M. Peter, L. Magnaghi-Jaulin, S. Vigneron, D. Loyaux, T. Lorca, J.C. Labbe, Part of *Xenopus* translin is localized in the centrosome during mitosis, Biochem. Biophys. Res. Commun. 276 (2000) 515–523.
[59] K.G. Manolis, E.R. Nimmo, E. Hartsuiker, A.M. Carr, P.A. Jeggo, R. Allshire, Novel functional requirements for non-homologous DNA end joining in *Schizosaccharomyces pombe*, EMBO J. 20 (2000) 210–221.
[60] D.K. Bishop, D. Zickler, Early decision: meiotic crossover interference prior to stable strand exchange and synapsis, Cell 117 (2004) 9–15.
[61] M.C. Whitby, Making crossovers during meiosis, Biochem. Soc. Trans. 33 (2005) 1451–1455.
[62] L.C. DeVeaux, G.R. Smith, Region-specific activators of meiotic recombination in *Schizosaccharomyces pombe*, Genes Dev. 8 (1994) 203–210.
[63] C.E. Pearson, K. Nichol Edamura, J.D. Cleary, Repeat instability: mechanisms of dynamic mutations, Nat. Rev. Genet. 6 (2005) 729–742.
[64] S.M. Woener, M. Kloor, M. Von Knebel Doeberitz, J.F. Gebert, Microsatellite instability in the development of DNA mismatch repair deficient tumors, Cancer Biomark. 2 (2006) 69–86.
[65] A.A. Mansour, C. Tornier, E. Lehmann, M. Darmon, O. Fleck, Control of GT repeat stability in *Schizosaccharomyces pombe* by mismatch repair factors, Genetics 158 (2001) 77–85.